Environmental and anthropogenic controls over bacterial communities in wetland soils

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Soil bacteria regulate wetland biogeochemical processes, yet little is known about controls over their distribution and abundance. Bacteria in North Carolina swamps and bogs differ greatly from Florida Everglades fens, where communities studied were unexpectedly similar along a nutrient enrichment gradient. Bacterial composition and diversity corresponded strongly with soil pH, land use, and restoration status, but less to nutrient concentrations, and not with wetland type or soil carbon. Surprisingly, wetland restoration decreased bacterial diversity, a response opposite to that in terrestrial ecosystems. Community level patterns were underlain by responses of a few taxa, especially the *Acidobacteria* and *Proteobacteria*, suggesting promise for bacterial indicators of restoration and trophic status.

16S rDNA | land use | phylogenetic analysis | restoration | soil pH

Soil bacterial communities play a critical role in regulating the cycling, retention, and release of major nutrients and soil carbon in freshwater wetlands, with demonstrably large effects on water quality (1) and global carbon cycling (2). However, little is known about the taxonomic composition of uncultured soil bacteria in freshwater wetlands relative to other ecosystems, despite the disproportionate influence of wetlands in controlling biogeochemical cycling at landscape scales (3). With a single exception in a *Sphagnum* bog (4), existing knowledge of bacterial communities in freshwater wetlands has been obtained using DNA fingerprinting (5, 6), group specific probes (7–9), or culture-based methods (8), which either have not identified bacterial taxonomic groups or do not adequately represent the vast diversity of uncultured soil bacteria (10). Furthermore, the environmental and anthropogenic factors controlling the distribution and abundance of bacterial groups in freshwater wetland soils are unknown.

To predict the effects of ecosystem change on wetland functions, improved understanding of the ecological responses of uncultured bacterial communities to ecosystem alteration is needed to compliment existing knowledge of bacterial functional groups controlling specific biogeochemical processes. The importance of understanding controls over wetland bacterial communities is underscored by the unique nature of wetlands as transitional ecosystems, the role wetland bacteria play in regulating biogeochemical fluxes across different ecosystem types, and increasing efforts to restore the functionality of degraded wetlands subjected to land-use change (11). In our unique study, we demonstrate the spectrum of uncultured bacterial communities across a range of freshwater wetland types and quantify the influence of soil chemistry, land use, restoration, and soil nutrient concentrations on bacterial assemblages.

Freshwater wetlands are transitional gradients between terrestrial and aquatic ecosystems, and thus may have environmental and anthropogenic controls over bacterial community structure similar to those of their neighboring ecosystems. Land use (12, 13) and soil chemistry (12, 14) have been shown to control microbial communities in several terrestrial systems. Ecosystem restoration has also been shown to alter microbial communities in terrestrial (15, 16) and wetland systems (5), although the specific phylogenetic groups of microbes affected by restoration have not yet been determined in either of these systems. Eutrophication and productivity gradients appear to be the primary determinants of microbial community composition in freshwater aquatic ecosystems (17, 18). To capture the range of likely controls over uncultured bacterial communities across freshwater wetland types, we chose sites representing a range of soil chemistry and land uses, including reference wetlands, agricultural and restored wetlands, and sites along a nutrient enrichment gradient.

The sites we selected represented a range of land uses encompassing natural, disturbed, and restored conditions across several freshwater wetland types, including pocosins (evergreen shrub bogs), riverine and nonriverine swamp forests, and calcareous fens. We determined the relative abundance of major phylogenetic groups of bacteria present (Fig. 1) and basic soil chemistry (Table S1) at nine sites in the North Carolina (NC) coastal plain (pH 3.5–6.0) and four sites in the Florida Everglades along a well-studied (3) nutrient enrichment gradient (pH 6.5–7.4, soil P concentrations ranging from 1,800 $mg.kg^{-1}$ to 350 $mg.kg^{-1}$). At each of the NC coastal plain wetland complexes we sampled soils from the following three land uses: (*i*) a wetland that had been converted to row crop agriculture; (*ii*) a restored wetland where ditches had been filled, tree seedlings had been planted, and natural vegetative recolonization had occurred; and (*iii*) a reference wetland representing conditions of the undisturbed ecosystem. We compared changes in the relative abundance of bacterial phylogenetic groups to soil chemistry (pH, % carbon, % nitrogen, and % phosphorus) and land-use categories.

Results and Discussion

The taxonomic composition of soil bacterial assemblages varied greatly between soils of NC coastal plain wetlands and the Florida Everglades, but much less within these two regions (Fig. 2). The bacterial groups present were similar among soils from pocosin bogs, and riverine and nonriverine swamp forests in the NC coastal plain, although the relative abundance of the groups present varied markedly. The composition abundance of dominant bacterial groups was unexpectedly uniform among soils collected along the Everglades nutrient-enrichment gradient, a result contrasting with observed shifts in the diversity of methanogenic *Archaea* along this gradient (7).

Bacterial communities in soils from NC coastal plain wetlands included diverse assemblages of bacterial phylogenetic groups (see Fig. 2), dominated by the *Acidobacteria* (mean 38.1% of

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The authors declare no conflict of interest.

Data deposition: The sequences reported in this paper have been deposited in the Genbank database (accession nos. EF443271–EF444484).

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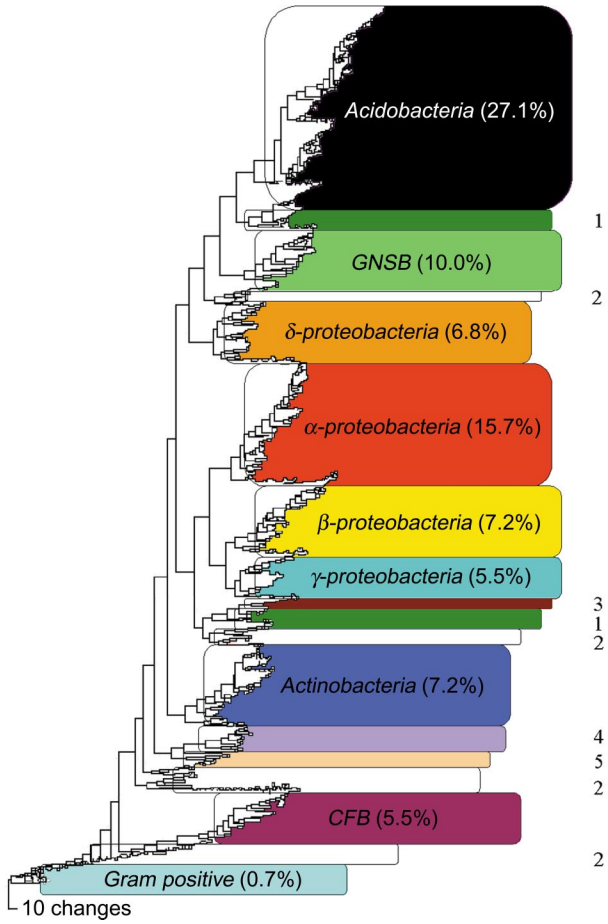

Fig. 1. Phylogenetic tree of 16S rDNA sequences obtained from freshwater wetland soils derived by parsimony analysis. Percent of total composition of major clades is given from 95 sequences at each of 13 sites. Minor clades are numbered: (*1*) *Nitrospira*, 2.6%; (*2*) *unknown*, 4.0%; (*3*) *Spirochaetes*, 1.3%; (*4*) *Prosectobacter*, 2.8%; (*5*) *Planctomycetes*, 1.0%. Not shown are *Gemmantimonas*, 0.7% and *Cyanobacteria*, 0.7%. Clade sizes are not directly proportionate to percent composition because of uneven inclusion of known guide sequences.

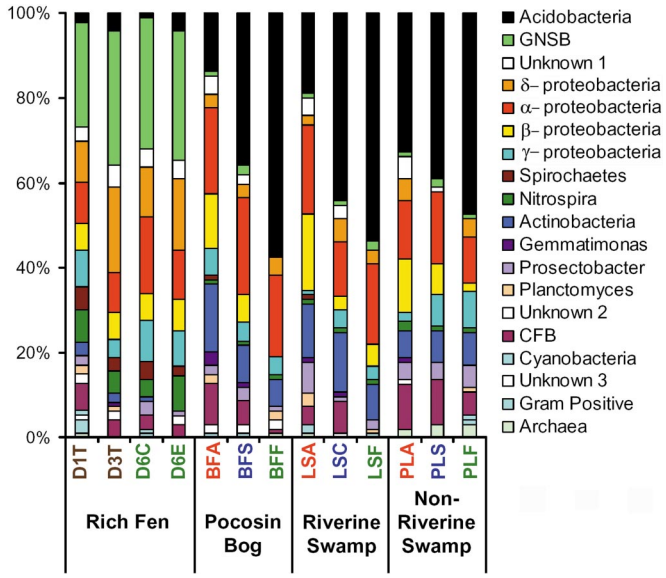

Fig. 2. Taxonomic composition of bacterial communities across different freshwater wetland ecosystem types and land uses. Taxonomic composition was determined by a phylogenetic tree of 95 clones of bacterial 16S rDNA from each site (Fig. 1). Site abbreviations are color coded by land use: nutrient enriched Everglades sites are brown, agricultural wetlands are red, restored wetlands are blue, and reference wetlands are green. Site abbreviations are described in detail in *Materials and Methods*.

clones), α- *proteobacteria* (17.4%), and *Actinobacteria* (9.7%). Other bacterial groups present included the *Cytophaga-Flavobacterium-Bacteriodies* (*CFB*) groups and the β-, δ-, and γ-divisions of the phylum *Proteobacteria*. These bacterial taxa together accounted for an average of 86.6% of the bacterial clones we obtained from soils of the North Carolina pocosin bogs, and riverine and nonriverine swamps.

Bacterial communities in soils along the Everglades nutrient-enrichment transect (see Fig. 2) were dominated by green nonsulfur bacteria (GNSB, mean 38.1% of clones), δ- *proteobacteria* (14.6%), and α- *proteobacteria* (12.2%). Other bacterial groups present included β- and γ- *proteobacteria*, *Nitrospira*, *CFB* groups, *Acidobacteria*, *Spirochaetes*, and an unknown bacterial clade. These bacterial groups accounted for 89.1% of the clones we obtained from soils along the Everglades nutrient-enrichment gradient.

Most of the bacterial groups present in our freshwater wetlands are widely distributed in surveys of uncultured microbial communities across terrestrial and aquatic ecosystems, although the composition of these groups varies across ecosystems (Table S2). The abundances of bacterial groups in our Everglades sites diverged the most from other ecosystems. Bacterial assemblages in the pocosin bogs we sampled were similar to those found in a *Sphagnum* bog (4). We used Mantel's tests to determine the independent influences of soil pH, land use, and nutrient concentrations on the distribution, abundance and diversity of bacterial taxonomic groups across several freshwater wetland types.

Bacterial community composition and diversity responded most strongly to soil pH across all of our wetland sites. Bacterial communities were highly correlated with soil pH ($r = 0.853$), even after accounting for the effects of wetland type, land use and restoration, and all other soil chemical variables using pure-partial Mantel's tests ($r = 0.747$) (Fig. 3). Soil pH also predicted diversity of bacterial phyla and "species" (97% sequence similarity operational taxomic units or OTUs) across all of our sites (Fig. 4 *A* and *B*). Effects of environmental pH on bacterial community composition and diversity have been recently noted in aquatic (19) and terrestrial ecosystems (20), respectively, although our present work is unique in linking pH with sequence-based changes in bacterial communities. We do not suspect shifts in bacterial communities with pH are methodological artifacts, as soil DNA-extraction efficiency does not vary with pH (21). Fittingly, we observed a strong increase in the abundance of *Acidobacteria* with lower pH (Fig. 4*C*), a relationship also found across terrestrial soils (22). The abundances of the *Actinobacteria* and *α-proteobacteria* had curvilinear relationships with soil pH (Fig. 4*D*), suggesting pH optima for these taxa.

Land use also significantly influenced bacterial community composition across wetland types. Bacterial assemblages clearly differed between wetland soils from the NC coastal plain and the Florida Everglades, and among coastal plain wetlands based on land use (Fig. 5). Land use predicted bacterial community composition across all of our wetland sites, even after accounting for wetland type and soil chemistry using pure-partial Mantel's tests (see Fig. 3*A*). Land-use change in upland systems has been shown to influence microbial community composition across disturbance gradients, ranging from agricultural fields to fallow grasslands to undisturbed grasslands (12, 13), and among different cultivation practices in agricultural fields (23).

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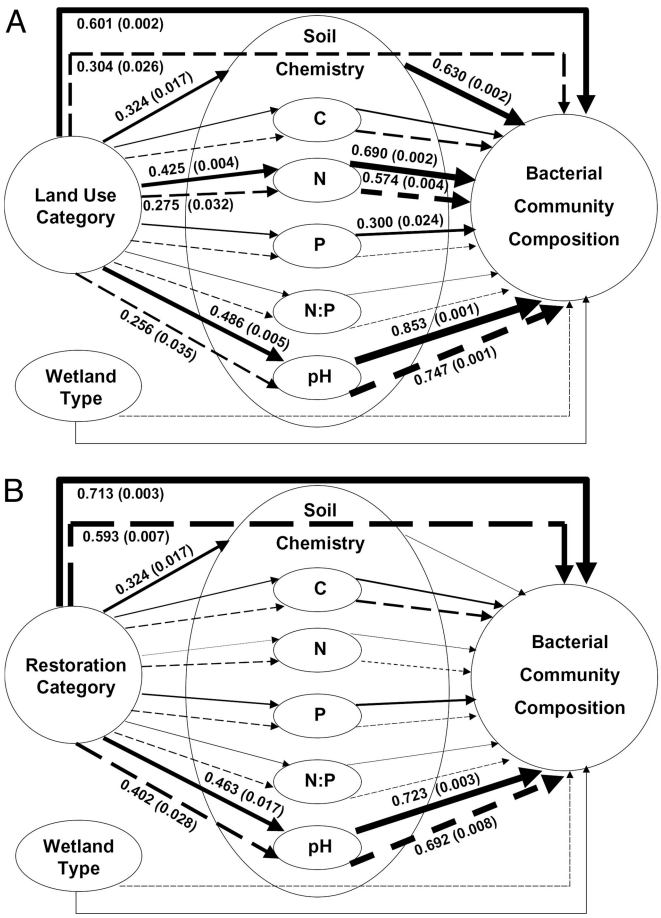

Fig. 3. Mantel path analysis linking taxonomic composition of microbial communities to soil chemistry, land use, and wetland type. (*A*) All wetland types surveyed, land use categories are: Everglades water conservation area (WCA), agriculture, restored, and reference. (*B*) North Carolina coastal plain wetlands were analyzed separately to determine effects of wetland restoration. Solid lines are partial Mantel correlation coefficients, while dashed lines are pure-partial Mantel correlation coefficients, conditional on all other variables. Where Mantel correlations are significant, line width is proportional to the correlation coefficient, and *P* values are in parentheses.

To determine the effects of wetland restoration on bacterial assemblages, we separately analyzed bacteria only in NC coastal plain soils, where restored sites could be compared with agricultural and reference wetlands within the same wetland type. Bacterial community composition was strongly related to wetland restoration category ($r = 0.713$), even after accounting for wetland type and soil chemistry using pure-partial Mantel's tests ($r = 0.593$) (see Fig. 3*B*). Bacterial diversity at both species and phyla levels was negatively correlated with wetland restoration, with significant differences among restoration categories at all NC coastal plain sites in Shannon's index (H')-based OTU accumulation ($P = 0.006$) and phylogenetic tree categories ($P = 0.001$) (data in Table S3). Wetland restoration also strongly influenced the normalized ratio of *Proteobacteia* to *Acidobacteria* (Fig. 6*A*), which is believed to reflect soil trophic status (24), and resulted in decreased abundance of the β-proteobacteria relative to agricultural soils (Fig. 6*B*).

Bacterial diversity of restored wetlands was intermediate between higher diversity agricultural soils and lower diversity reference wetlands at all of our NC coastal plains sites (Fig. 7), a result opposite of that found in restoration of terrestrial ecosystems, where reference soils have the most diverse bacterial communities (15, 16). However, our soils were restored from agricultural fields rather than spoils (16), and unlike more neutral soils (15), were likely limed as well as fertilized. Liming has also been shown to affect microbial communities in acidic grassland soils, although by decreasing rather than increasing diversity (14). Wetland restoration generally represents a return to less fertile soil conditions, characterized by partial recovery of acidity and anoxia in soils following the cessation of liming and fertilization, and increased flooding (25), which may limit the diversity of bacteria by increasing metabolic stresses. Suitably, the lower bacterial diversity in our reference wetland soils appears to be related to increased dominance of the *Acidobacteria* in less-disturbed wetlands (see Figs. 2 and 5).

We found some correspondences between soil nutrient concentrations and bacterial communities of wetland soils. Soil nitrogen and phosphorus concentrations were correlated with bacterial community composition across all wetland types (see Fig. 3*A*). However, soil nutrient concentrations did not predict bacterial community composition in wetland soils of the NC coastal plain (see Fig. 3*B*), and there was little difference in bacterial community composition along the Florida Everglades nutrient-enrichment transect (see Figs. 2 and 5).

Weaker relationships between nutrients and bacterial communities we observed at local scales may suggest regional scale relationships are the result of high nutrient concentrations and distinct bacterial communities in Everglades soils (see Fig. 5). Although microbial communities reflect trophic status in aquatic ecosystems (17, 18), we expect the response of microbial communities in wetland soils to be less pronounced as a result of the predominance of soil-bound nutrients in wetlands (3), as microbial communities often do not correspond to soil nutrient status in terrestrial soils (12, 24). Stronger relationships between bacterial communities and nutrients in wetlands may also result from analysis of available nutrient pools instead of total nutrient concentrations in future studies.

Our findings demonstrate responses of bacterial communities to environmental and anthropogenic gradients in wetland soils, and we emphasize a comparative approach with terrestrial and aquatic ecosystems. Our approach linking biogeography to ecosystem change is complimentary with studies that seek to determine bacterial functional groups (26). Although specific bacterial groups have been linked to biogeochemical cycles in wetlands [e.g. (7–9)], structure-function relationships vary in degree and kind with biogeochemical process, and element cycling may be affected by previously unknown organisms (27). We emphasize that understanding controls over the distribution and abundance of uncultured bacterial communities is a required first step in determining structure-function relationships that compliment attempts to delineate functional guilds (28). Our approach also addresses the lack of prior knowledge of the composition and controls over uncultured bacterial communities in freshwater wetlands, and the impact of environmental change on these ecosystems, which may alter both bacterial community structure and function.

Our results reveal shifts in the composition of whole bacterial communities, and the abundance of specific taxonomic groups with environmental gradients that may reflect changes in biogeochemical cycling. Soil pH broadly altered the composition and diversity of our wetland soils and affected specific taxa, including the *Acidobacteria*, *Actinobacteria*, and *α-proteobacteria*. Soil pH also alters bacterial growth and biogeochemical process rates in mixed peat-bog cultures (8) and controls degradation of lignocellulose in wetlands (29). The effect of pH on decomposition might be mediated by shifts in bacterial composition with pH, as the acidophillic *Acidobacteria* are oligotrophs characterized by slow growth rates and metabolism of more refractory carbon substrates characteristic of peat soils (22). Analogously, we observed a greater abundance of the *β-proteobacteria* in agricultural soils (see Fig. 6*B*), and shifts in their abundance

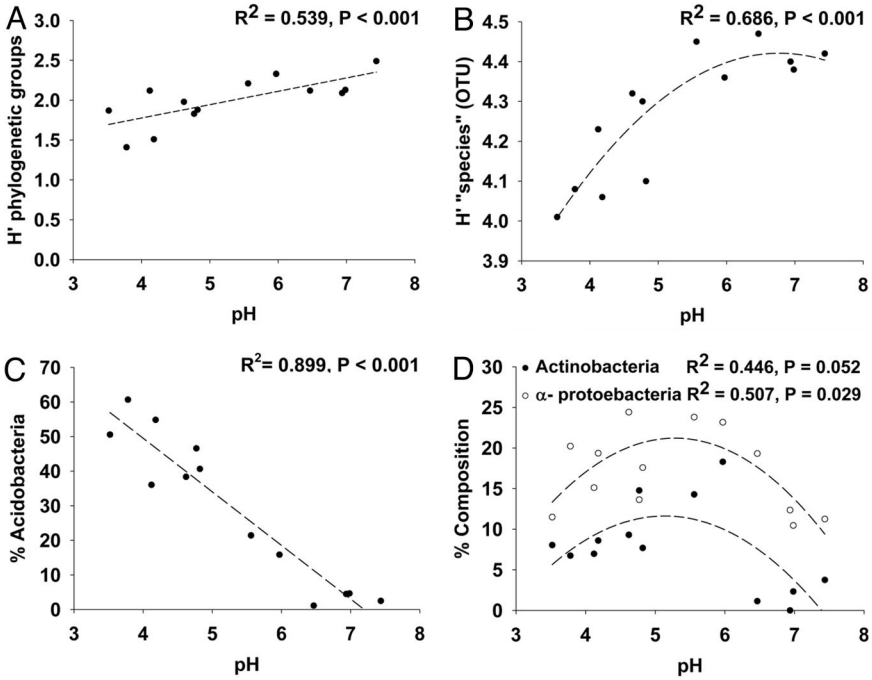

Fig. 4. Soil pH controls bacterial diversity and the relative abundances of select bacterial taxa. Soil pH influences bacterial diversity as assessed by Shannon's index (H') of (*A*) phylogenetic groups derived from Fig. 1, and (*B*) species level OTUs, assessed at 97% similarity and derived from Fig. 4. Soil pH also determines the abundance of some bacterial taxa, including (*C*) *Acidobacteria*, and (*D*) *Actinobacteria*, and α-*proteobacteria*.

along restoration gradients (see Fig. 5), suggesting an important response to land-use change. The *β-proteobacteria* increase in abundance with eutrophication in aquatic ecosystems (18), and are capable of ammonium oxidation (9, 27), denitrification, and polyphosphate accumulation (27), indicating an important role of this group in nutrient cycling in eutrophic ecosystems. Changes in the abundance of bacterial groups may be readily indexed, as we found wetland restoration altered the normalized ratio of *Proteobacteria* to *Acidobacteria* (see Fig. 6*A*), which has been suggested as a broad indicator of trophic status across a range of terrestrial soils (24).

We discovered that the composition, relative abundance, and diversity of bacterial groups in wetland soils were determined by soil pH, land use, and restoration. While relationships between soil pH and bacterial communities were consistent with those in aquatic (19) and terrestrial ecosystems (20), wetland restoration resulted in decreased bacterial diversity, a finding opposite of results obtained in terrestrial soils (15, 16). Relationships between nutrient concentrations and wetland bacterial communities were less robust than those in aquatic systems, but not unlike

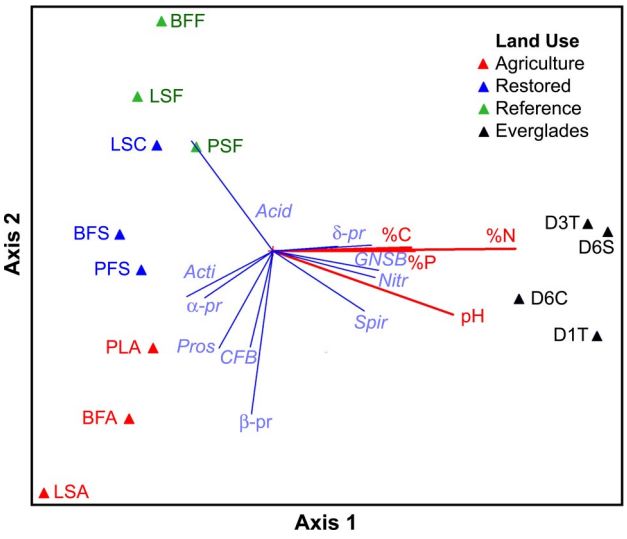

Fig. 5. Bacterial community ordination by land use, based upon the relative abundance of bacterial taxonomic groups, by principal components analysis. Axis 1 explains 52.6% of variance, while Axis 2 describes an additional 19.2% of variance among samples. Factor loadings are shown with blue vectors for taxonomic groups with >20% loading. Bacterial group abbreviations (clockwise from top) are *Acid*: Acidobacteria; δ-*pr*: δ-proteobacteria; *GNSB*: Green Non Sulfur Bacteria; *Nitr*: Nitrospira; *Spir*: Spirochaetes; β-*pr*: β-proteobacteria; *CFB*: Cytophaga-Flavobacterium-Bacteriodies; *Pros*: Prosectobacter; α-*pr*: α-proteobacteria; *Acti*: Actinobacteria. Site abbreviations are described in detail in *Materials and Methods*.

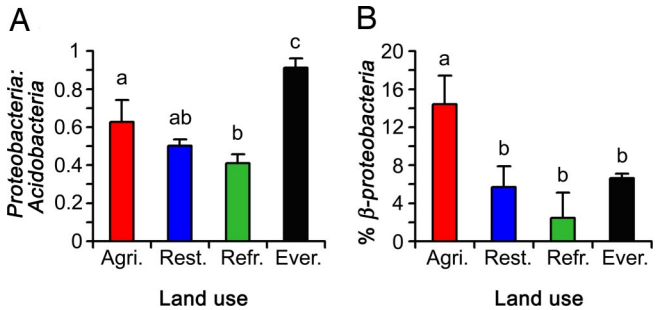

Fig. 6. Abundance of bacterial taxonomic groups varies with land use across wetland soils. Italicized letters indicate statistical differences determined by Tukey's multiple comparisons. (*A*) Land use altered the normalized ratio of all *Proteobacteria* to *Acidbacteria* with land use ($P < 0.001$). (*B*) the abundance of *β-proteobacteria* was greater in agricultural wetlands than in other land uses ($P < 0.001$). Statistical grouping of sites was the same for the normalized ratio of *β-proteobacteria* to *Acidobacteria* as for the ratio of all *Proteobacteria* to *Acidbacteria* ($P < 0.001$, data in Table S3).

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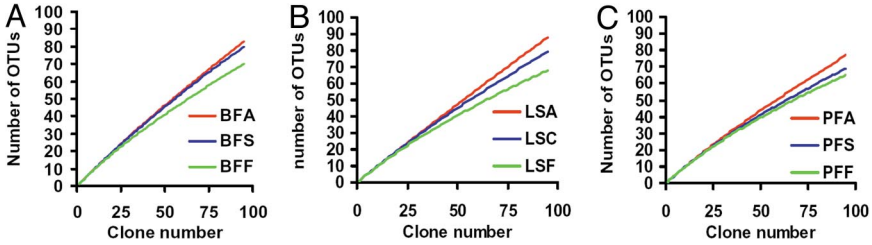

Fig. 7. Soil bacterial diversity shifts with land use and restoration across NC wetland types. Collector's curves present the number of unique bacterial species (defined at 97%) obtained from a given site, called OTUs. Restoration land use categories are agriculture (*red*), restored (*blue*), and reference wetlands (*green*). Wetland types are (*A*) pocosin bogs, (*B*) riverine swamp forests, and (*C*) nonriverine swamp forests. Site abbreviations are described in detail in *Materials and Methods*.

results in terrestrial systems. Further cross-system comparisons of bacterial communities and environmental gradients may reveal emergent properties across ecosystems, like those linking terrestrial and aquatic biogeochemistry (30). Our findings may also have more immediate implications, as we demonstrate bacterial indicators that may be applied to wetland restoration and management, like those suggested for terrestrial (31) and aquatic ecosystems (32).

Materials and Methods

Site Descriptions. Soil samples were collected in the fall of 2003 from a range of wetland sites along gradients of differing land use history in the North Carolina coastal plain, and along a eutrophication gradient in the Florida Everglades. We sampled three NC coastal plain locations that each had agricultural wetlands, restored wetlands, and reference wetlands in close proximity: Barra Farms, Long Swamp, and Parker Farms.

The Barra Farms site (BF) is part of a 975-ha Carolina bay complex located in Cumberland County, North Carolina (25). Soils at the site have been classified as Croatan mucks (Terric Haplosaprists). Past alterations to the site included clearing and ditching in the 1960s for conversion to agriculture, and intensive farming during the 1970s and 80s. In the fall of 1997, 250 ha of the site were restored to wetland by filling ditches and planting woody seedlings. Samples were obtained from existing agricultural soils (BFA), from the 6-year-old restored area (BFS), and from a reference site in a nonriverine swamp forest section of the site that was never converted to agriculture (BFF).

Long Swamp (LS) is a 10-ha site located in Hoke County, North Carolina. The soils at the site have been classified as Johnston loams (Cumulic Humaquepts) and Rains loamy sands (Typic Paleaquults). The site is located in a flat, forested headwater area of LS stream. Past alterations of the site include clearing and ditching for conversion to agriculture, as well as timber harvesting. The site was restored in 1998 by filling in ditches and planting woody seedlings. Soils were collected in restored areas that had been impacted by agriculture (LSA) and a 5-year-old forest clearing (LSC), as well as from a reference forested section of the site that had not been previously cleared (LSF).

Parker Farms (PF) is a 160-ha site located in Beaufort County, North Carolina (30). The soils at the site have been classified as Wasda mucks (Histic Humaquepts) and Ponzer mucks (Terric Haplosaprists). This site was originally a nonriverine swamp forest that was cleared, ditched, and converted to agriculture. In 1995 the site was restored by filling ditches and planting with woody seedlings. Samples were collected from a nearby agricultural field with Terric Haplosaprist soils that had just been incorporated into the Pocosin Lakes National Wildlife Refuge (PFA), as well as from the 8-year-old restored area of Parker Farms (PFS), and a reference wetland on the Parker Farms tract that had never been cleared (PFF) (33).

The Florida Everglades is part of an ongoing study along a 40-year nutrient-enrichment gradient in the northern part of the subtropical Everglades (26° 15′ N, 80° 23′ W). Surface-water and soil P has been shown to be elevated above natural, background concentrations up to 7 km into the interior of WCA-2A (34, 35). Soils were collected at 1, 3, and 6 km from the D water control structure in WCA-2A, along a well-studied nutrient-enrichment gradient that declines in intensity moving away from the water control structure. Plant communities were dominated by *Typha domingensis* at 1 and 3 km along the gradient (D1T and D3T, respectively), while at 6 km, samples were collected from areas dominated by *Cladium jamaicense* (D6C) and from open sloughs colonized by *Eleocharis elongata (*D6E).

Soil Collection and Analyses. At each sampling location, the top 10 cm of soil was collected from three points within a 5-m radius. Soils were sieved wet and replicate samples were pooled and homogenized. Soil organic matter was determined by loss on ignition, total N was determined by carbon, hydrogen, nitrogen (CHN) analysis, total P was determined by Murphy Riley following a perchloric acid digest (36), and pH was determined in 1:1 soil:water slurries.

Bacterial 16S rDNA Sequencing and Analysis. Soil DNA was extracted using an Ultra Clean MoBio soil DNA extraction kit (MoBio Labs). Extracted DNA was amplified using bacterial specific 16S rDNA primers BSF 343/15 (TACGGRAGGCAG) and BSR 926/20 (CCGTCAATTYTTTRAGTT), which amplify a *ca.* 560-bp fragment (37). DNA was amplified by PCR with an initial denaturation step of 94 °C for 3 min, followed by 25 cycles of 94 °C for 1 min, 50 °C for 30 s, and 72 °C for 2 min, and a final annealing step at 72 °C for 7 min. Ninety-five clones were obtained from each soil sample by cloning amplified DNA using a TOPO TA cloning kit (Invitrogen Corp.). Individual clone colonies were amplified by PCR using dentaturation at 94 °C for 10 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final annealing step at 72 °C for 10 min. Clone PCR products were purified using a Qiagen PCR purification kit (Qiagen, Inc.). Amplified clone DNA was sequenced using ABI BigDye (Applied Biosystems, Inc.) on an ABI 3700 capillary DNA sequencer. Sequences were deposited in GenBank under accession numbers EF443271–EF444484.

Microbial DNA sequences identified were compared to NCBI Blast (38) and RDP sequence classifier databases (39) for identification, with only close matches ($>$ 98%) accepted for identification. Only about 15% of sequences were identified with database matches. Poorly matching sequences ($<$ 65% identity) were screened for chimeric recombination using RDP Chimera Checker (35). OTUs at 97% sequence similarity (40) were obtained for each using Sequencher (Gene Codes, Inc.). Phylogenetic identities of unknown sequences were determined by creating a phylogenetic tree of sequences in our clone library (Fig. 1) using parsimony analysis in PAUP (Sinauer Assoc., Inc.). Maximum likelihood analyses were not used because of our large clone library ($>$ 1,300 sequences). Phylogenetic identities of unidentified sequences were assigned at phyla or class levels by comparing clade positions to sequences identified by BLAST and RDP, and known sequences from a database of 218 16S sequences representing major bacterial groups obtained from RDP (39).

Data Analysis. Microbial diversity was calculated from both OTU data and phylogenetic data by obtaining Shannon's index (H′) using EstimateS (41). Microbial diversity (H′) was compared to soil C, N, P, and pH using simple linear regression in S-PLUS (Version 6.2, Insightful Software, Inc.). Phylogenetic data on microbial community composition at each site was compared to soil chemical parameters (C, N, P, and pH) using partial and pure-partial Mantels' tests (42–44) of Euclidean distance matrices. The Mantel test procedure was carried out in S-PLUS using code developed by S. Goslee (45). The significance of the Mantel correlation was assessed by permutation, as the elements of these matrices are not independent (46). Significance of the coefficients was estimated by bootstrapping with 1,000 random permutations. Mantel correlation coefficients do not behave like product-moment correlation coefficients, and do not have to be large in absolute value to be statistically significant (47). Path diagrams (48) were created as a visual framework for examining the correlations among bacterial community composition, land use, and soil chemistry. Ordination of bacterial communities was performed using principal components analysis (PCA) of the relative abundance of different taxonomic groups compared to our soils data using PC ORD 5 (MjM software design). We also used UNIFRAC (49) to compare bacterial communities among sites and land-use treatments, and results from UNIFRAC ordination were nearly identical to those obtained by PCA. We decided to

use results from PCA ordination based upon the relative abundance of taxonomic groups rather than sequence-based distance from UNIFRAC because 16S rRNA sequence phylogeny does not accurately represent bacterial taxonomy, but rather is useful as a taxonomic marker to be compared to known sequence phylogeny, as in our approach using relative abundance of taxonomic groups determined by phylogenetic relationships to a guide tree of known organisms.

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1. Richardson CJ, Marshall PE (1986) Processes controlling the movement, storage, and export of phosphorus in a fen peatland. *Ecol Monogr* 56:279–302.
2. Roulet NT (2000) Peatlands, carbon storage, greenhouse gases and the Kyoto Protocol: Prospects and significance for Canada. *Wetlands* 20:605–615.
3. Richardson CJ (2008) *The Everglades Experiments: Lessons for Ecosystem Restoration* (Springer, New York) p 698.
4. Dedysh SN, Pankratov TA, Belova SE, Kulichevskaya IS, Liesack W (2006) Phylogenetic analysis and in situ identification of *Bacteria* community composition in an acidic *Sphagnum* peat bog. *Appl Env Microbiol* 72:2110–2117.
5. D'Angelo EM, Karathanasis AD, Sparks JE, Ritchey SA, Wehr-McChesney SA (2005) Soil carbon and microbial communities at mitigated and late successional bottomland forest wetlands. *Wetlands* 25:162–175.
6. Angeloni NL, Jankowski KJ, Tuchman NC, Kelly JJ (2006) Effects of an invasive cattail species (*Typha x glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland. *FEMS Microbiol Lett* 263:86–92.
7. Castro H, Ogram A, Reddy KR (2004) Phylogenetic characterization of methanogenic assemblages in eutrophic and oligotrophic areas of the Florida Everglades. *Appl Env Microbiol* 70:6559–6568.
8. Sizova MV, Panikov N, Tourova TP, Flanagan PW (2003) Isolation and characterization of oligotrophic acido-tolerant methanogenic consortia from a Sphagnum peat bog. *FEMS Microb Ecol* 45:301–315.
9. Kowalchuck GA, Bodelier PLE, Heilig GHJ, Stephen JR, Laanbroek HJ (1998) Community analysis of ammonia-oxidising bacteria in relation to oxygen-availability in soils and root-oxygenated sediments, using PCR, DGGE and oligonucleotide probe hybridisation. *FEMS Microb Ecol* 27:339–350.
10. Tiedje JM, Asuming-Brempong S, Nusslein K, Marsh T, Flynn SJ (1999) Opening the black box of soil microbial diversity. *Appl Soil Ecol* 13:109–122.
11. Mitsch WJ, Gosselink JG (2000) *Wetlands* (Van Nostrand Reinhold, New York) 3rd Ed.
12. McCaig AE, Glover A, Prosser JI (2001) Numerical analysis of grassland bacterial community structure under different land management regimens by using 16S ribosomal DNA sequence data and denaturing gradient gel electrophoresis banding patterns. *Appl Env Microbiol* 67:4554–4559.
13. Buckely DH, Schmidt TM (2003) Diversity and dynamics of microbial communities in soils from agroecosystems. *Env Microbiol* 5:441–452.
14. Kennedy N, Brodie E, Connolloy J, Clipson N (2004) Impact of lime, nitrogen and plant species on bacterial community structure in grassland microcosms. *Env Microbiol* 6:1070–1080.
15. McKinley VL, Peacock AD, White DC (2005) Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. *Soil Biol Biochem* 37:1946–1958.
16. DeGrood SH, Claassen VP, Scow KM (2005) Microbial community composition on native and drastically disturbed serpentine soils. *Soil Biol Biochem* 37:1427–1435.
17. Rajendran N, Matsuda O, Radjendran R, Urushigawa Y (1997) Comparative description of microbial community structure in surface sediments of eutrophic bays. *Mar Pollut Bull* 34:26–33.
18. Wobus A, *et al.* (2003) Microbial diversity and functional characterization of sediments from reservoirs of different trophic states. *FEMS Microb Ecol* 46:331–347.
19. Lindstrom ES, Kamst-Van Agterveld MP, Zwart G, (2005) Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. *Appl Env Microbiol* 71:8201–8206.
20. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* 103:626–631.
21. Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. *Appl Env Microbiol* 62:316–322.
22. Eichorst SA, Breznak JA, Schmidt TM (2007) Isolation and characterization of soil bacteria that define *Terriglobus* gen. nov., in the Phylum *Acidbacteria. Appl Env Microbiol* 73:2708–2717.
23. Girvan MS, Bullimore J, Petty JN, Osborn AM, Ball AS (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Appl Env Microbiol* 69:1800–1809.
24. Smit E, *et al.* (2001) Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl Env Microbiol* 67:2284–2291.
25. Bruland GL, Hanchey MF, Richardson CJ (2003) Effects of agriculture and wetland restoration on hydrology, soils, and water quality of a Carolina bay complex. *Wetl Ecol Mgmt* 11:141–156.
26. Zak DR, Blackwood CB, Waldrop MR (2006) A molecular dawn for biogeochemistry. *Trends Ecol Evol* 21:288–295.
27. Wagner M, Loy A (2002) Bacterial community composition and function in sewage treatment systems. *Curr Opin Microbiol* 13:218–227.
28. Naeem S, Wright JP (2003) Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. *Ecol Lett* 6:567–579.
29. Benner R, Moran MA, Hudson RE (1985) Effects of pH and plant source on lignocellulose biodegradation rates in two wetland ecosystems, the Okefenokee Swamp and a Georgia salt marsh. *Limnol Oceanogr* 30:489–499.
30. Grimm NB, *et al.* (2003) Merging aquatic and terrestrial perspectives of nutrient biogeochemistry. *Oecologia* 137:485–501.
31. Harris JA (2003) Measurements of the soil microbial community for estimating the success of restoration. *Eur J Soil Sci* 54:801–808.
32. Paerl H, *et al.* (2003) Microbial indicators of aquatic ecosystem change: Current applications to eutrophication studies. *FEMS Microb Ecol* 46:233–246.
33. Bruland GL, Richardson CJ (2006) Comparison of soil organic matter in created, restored and paired natural wetlands in North Carolina. *Wetl Ecol Mgmt* 14:245–251.
34. DeBusk WF, Reddy KR, Koch MS, Wang YF (1994) Spatial distribution of soil nutrients in a northern Everglades marsh: Water Conservation Area 2A. *Soil Sci Soc Am J* 58:543–552.
35. McCormick PV, Rawlik PS, Lurding K, Smith EP, Sklar FH (1996) Periphyton-water quality relationships along a nutrient gradient in the northern Florida Everglades. *J N Am Benth Soc* 15:433–449.
36. O'Halloran IPO (1993) in *Soil Sampling and Methods of Analysis*, ed Carter MR (Lewis Publishers, Boca Raton, FL) pp 213–229.
37. Wilmotte A, Van Der Auwera G, DeWachter R (1993) Structure of the 16S ribosomal RNA of the thermophilic cyanobacterium *Chlorogloeopsis* HTF (*Mastigocladus laminosus* HTF) strain PCC7518, and phylogenetic analysis. *FEBS Lett* 317:96–100.
38. Benson DA, *et al.* (2000) GenBank. *Nucleic Acids Res* 28:15–18.
39. Maidak BL, *et al.* (2001) The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* 29:173–174.
40. Stackebrandt E, Goebel BM (1994) Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bact* 44:846–849.
41. Colwell RK, Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation. *Phil Trans Royal Soc London B* 345:101–118.
42. Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209–220.
43. Sanderson RA, Rushton SP, Cherrill AJ, Byrne JP (1995) Soil, vegetation and space: An analysis of their effects on the invertebrate communities of a moorland in North-East England. *J Appl Ecol* 32:506–518.
44. King RS, Richardson CJ, Urban DL, Romanowicz EA (2004) Spatial dependency of vegetation-environment linkages in an anthropogenically influenced wetland ecosystem. *Ecosystems* 7:75–97.
45. Urban DL, Goslee S, Pierce K, Lookingbill T (2002) Extending community ecology to landscapes. *Ecoscience* 9:200–212.
46. Manly B (1997). *Randomization, bootstrap, and Monte Carlo methods in biology*, 2nd Ed. (Chapman and Hall, London).
47. Legendre P, Fortin MJ (1989) Spatial pattern and ecological analysis. *Vegetatio* 80:107–138.
48. Leduc A, Drapeau P, Bergeron Y, Legendre P (1992) Study of spatial components of forest cover using partial Mantel tests and path analysis. *J Veg Sci* 3:69–78.
49. Lozupone C, Knight R (2005) UniFrac: A new phylogenetic method for comparing microbial communities. *Appl Env Microbiol* 71:8228–8235.